(54) Title: METHOD AND APPARATUS FOR SELECTIVELY RETRIEVING BIOLOGICAL SAMPLES FOR PROCESSING
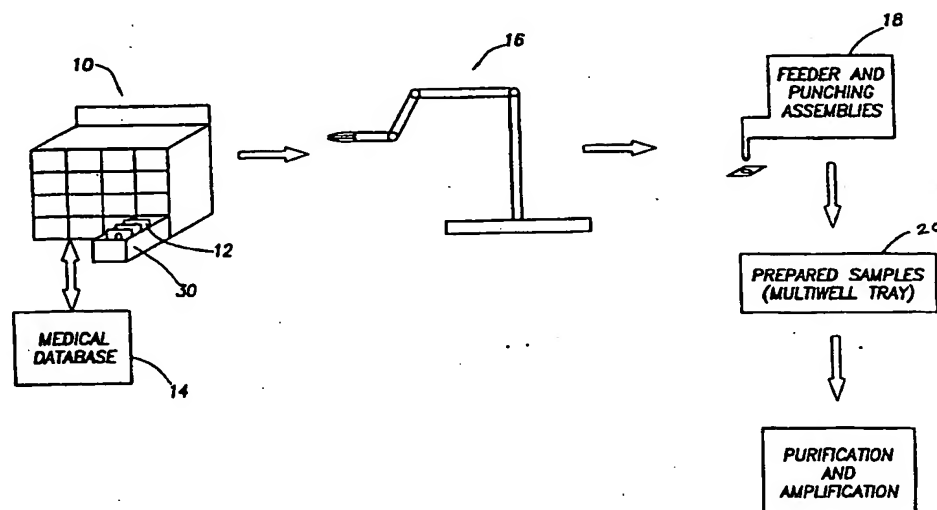
(57) Abstract: Biological retrieval system employing a DNA biological specimen repository (10) and robotic mechanism (16) for automated retrieval and processing of the individual specimens. The DNA repository (10) includes individual specimens which include an identification code (8) readable by the robotic assembly (16) and correlated with medical information about the individual whose specimen is on file. The medical information is housed within a database (14). Upon request, a population sample is determined from the database and the robotic system (16) retrieves particular specimens for subsequent processing. Selected specimens are delivered to a first staging area. At that point, a feeder assembly (34) relocates the biological specimens to a second staging area for punching by a punch head assembly (72). The punch head assembly (72) moves each pellet or punched sample to a predetermined location, such as for example, a single well of a multiwell tray (20) for subsequent biological processing.

METHOD AND APPARATUS FOR SELECTIVELY RETRIEVING BIOLOGICAL SAMPLES FOR PROCESSING

FIELD OF THE INVENTION

This invention relates to a method and apparatus for selectively retrieving biological samples for processing. More particularly, this invention relates to a DNA biological repository and the selection of specific biological specimens from the repository for subsequent processing.

BACKGROUND

5   The related fields of pharmacogenomics and genetic epidemiology have matured rapidly as spin-offs from the human genome project. Single nucleotide polymorphism (SNP) data is accumulating at a rapid pace due to re-sequencing of the human genome. Large-scale SNP discovery initiatives in the U.S. and Japan are defining high variability in the genetic make-up of the human population at the nucleotide level.

10   Such large-scale genetic projects require the study of gene polymorphism in very large human sample sets, as large as 100,000 to 500,000, in a manner that allows rapid, random access to genetic material from such samples at rates on the order of thousands per day.

As a result of managing such large sample libraries, a bottleneck has developed relative to the long-term storage of DNA samples and rapid, random-access retrieval of DNA from such

15   libraries. It is therefore desirable to provide technology supporting high-throughput genotyping that includes the permanent storage and indexing of such samples and rapid, addressable and substantially automatic processing of the genetic material in such samples.

SUMMARY OF THE INVENTION

Briefly, the invention is a biological retrieval system having a repository of biological

20   specimens. A robotic mechanism is provided for retrieving predetermined specimens based on an identification code associated with each specimen, the identification of which particular specimen to retrieve is determined from a database. The robotic mechanism delivers the selected specimens to a first staging area. A feeder assembly retrieves such specimens from the first staging area and removes specific specimens for delivery to a second staging area. At the second staging area, a

small sample is punched or removed from each specimen thereby providing the biological sample. Each biological sample is then delivered to a third staging area which may be, for example, a multiwell tray assembly. Each biological sample is then deposited in one well of the multiwell tray and is thereby uniquely associated with a particular individual whose medical data is on the database. The samples are then available for subsequent processing, such as purification and amplification, and then for genotyping, genoexpressing, or other biological processing. The robotic mechanism also returns the retrieved specimen from the first staging area back to the repository.

The present invention also includes an apparatus associated with removing a biological sample from the substrate of each biological specimen. This apparatus includes a feeder assembly which retrieves the specimen from the second staging area and delivers each individual specimen to a punching plate, also referred to as the second staging area. A punch head assembly of the apparatus removes a small biological sample from the substrate of each specimen. The punch head includes a tip to punch a pellet from the specimen, a reservoir to retain the pellet while it is transferred from second staging area to the third staging area. A position controller is included to selectively position the reservoir containing the pellet over a particular spot, or well of the multiwell tray, the third staging area. The punch head also includes an injector to remove the pellet and deposit it in the precise well. Alternatively, the retrieval of the pellet may be performed by a laser cutting system rather than a mechanical punch. However, the delivery is the same.

In practicing the method of the present invention, one first identifies, using a database, particular specimens to be retrieved from the DNA biological repository. Such specimens are then retrieved and delivered to the first staging area. The retrieved specimens are then taken from the first staging area and delivered individually or as a predetermined grouping to a second staging wherein, on an individual basis, a biological sample is removed from each specimen. That biological sample is then delivered to a third staging area for subsequent processing. Such subsequent processing may include purification of the sample and then amplification using standard PCR techniques. Preferably, each sample is delivered to a particular location at the third staging area, such as a particular well of the multiwell tray, which uniquely associates the DNA biological sample deposited in each well with a particular individual whose medical history can be found and correlated on the database. Following amplification the DNA samples may be used in genotyping or genoexpressing, for example, as disclosed and claimed in pending U.S. patent application serial no. 09/217,154, which application is hereby incorporated by reference.

of "real estate" on the blood specimen have already been punched since each specimen is bar coded and microprocessor 152 recalls which locations have been previously punched from a given specimen 12 or substrate 24. To do this, microprocessor 152 is connected to a slide database 154. Each indexed slide 12 has a particular number of "punchable" positions, each having an x and y coordinate stored in database 154. In this manner, prior to punching, microprocessor 152 working with digital camera 110 can inform the operator and microprocessor 100 that a particular slide, identified by its bar code for example, only has space remaining for punching at a particular spot. Thus, the positioning of the punching head on that slide is determined from historical data with each individual blood spot being arranged in a virtual grid.

In the operation of the present invention, an operator or customer performs a search of medical database 14 determining a population sample to study. The identification of particular DNA specimens are then identified and provided to a robotic system 16 which retrieves either individual specimens 12 from the DNA repository 10 or complete drawers or racks 30 containing one or more selected DNA specimens. The robotic system 16 deposits the retrieved specimens in a first staging area 32. A feeder assembly 34 then retrieves the individual drawers 30 which contain either a collection of specimens to be tested or only specimens to-be-selected from each drawer. Each drawer is then rotated by arms 33/35 of feeder assembly 34 from a horizontal attitude to a vertical attitude. Knowing which particular samples are to be punched, the operator initiates drive motor 62 which vertically displaces drawer 30A to a predetermined location. Activation of cylinder 44 advances plate 50 and, in turn, a given specimen 12 from the vertically oriented drawer 30A onto punching plate 56. The operator has pre-programmed microprocessors 110/152 informing each of the particular specimen to be tested. Since microprocessor 152/database 154 know the location of remaining "real estate" on a given specimen, it directs punch head 76 to a precise location on the specimen. Rapid movement in a "z" or vertical direction at the direction of microprocessor 100 causes punch head 76 to pierce substrate 24 dislodging a pellet 100 from the substrate into reservoir 80 of head 76. This is possible because arm 66 is mounted on frame 64 enabling rapid vertical descent (i.e., in the z-axis). Microprocessor 100 then instructs the movement of arm 66 along x, y and z axes to a particular location above a particular well 72 of tray 20. Solenoid 90 is then activated which disposes that particular pellet from reservoir 80 into a particular well 72 and also cleans bore 88 of the head 76 as it ejects the pellet. After a pellet 100 is removed from a particular substrate 24, cylinder 44 is activated returning that particular specimen 22 to its previous

location in tray 30A. In this manner, moving a particular specimen 22 from punching plate 56 and the delivery of another specimen 22 to punching plate 56 can occur while punching head 76 is positioning a particular pellet 100 into a specific well of tray 20.

5   Alternatively, a laser embodiment may be used rather than a mechanical punching assembly. Such laser techniques are well known to those skilled in the art and essentially involve the use of CO_2 lasers to cut a pellet from a substrate in a donut configuration and deposit that pellet in a particular well of a tray 20 using a vacuum to draw the pellet within the tip of a laser and then ejecting the pellet into a particular well by removing the vacuum. Preferably, such lasers are CO_2 vacuum lasers such as those manufactured by Synrad, Inc. of Mukilteo, Washington.

10   Once the required number of DNA specimens have been removed to fill, or partially fill, a tray 20 as required by a particular operation, tray 20 may be removed and then processed using conventional purification and amplification techniques, such as PCR, for subsequent biological testing or assay. Such assays may include genotyping and gene expression assay. In this manner, the present invention may be used to sample thousands of particular DNA specimens on a daily basis

15   significantly increasing the volume of throughput capacity for subsequent DNA biological processing.

After a sample has been removed by the punch head, the feeder assembly 34 returns the specimen 22 to drawer 30A as described above and the feeder assembly then returns each drawer 30A to the first staging area 32. Robot 16 then returns each individual drawer 30 to the repository

20   10. Thus, the present invention provides for a plurality of biological specimens 22 stored within the repository 10 in such a manner that they can be selectively retrieved and returned for multiple use due to the plurality of sample space available on each biological specimen. The selective retrieval and returning of such specimens and their reusable nature is a significant benefit of the present invention, particularly when combined with the other attributes of the feeder assembly and the punch

25   head assembly to accomplish the stated objections of the present invention.

As noted above, the foregoing has been described in terms of a blood sample being deposited on a substrate. Obviously, any number of other biological specimens may be used other than blood such as blood serum, blood plasma, blood lymphocytes, fixed or unfixed tissue extracts, buccal scrapes, DNA, RNA or protein.

A preferred embodiment of the invention has been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention as disclosed and claimed.

CLAIMS

1.   A biological retrieval and processing system comprising:

a repository of biological specimens;

a robot for retrieving predetermined specimens from said repository and delivering said retrieved specimens to a first staging area and for returning each specimen from said first staging area back to said repository;

a feeder adapted to retrieve said specimens from said first staging area and deliver each retrieved specimen to a second staging area; and

a punch head operatively associated with said feeder adapted to remove a sample from a substrate of each retrieval specimen and deliver each said sample to a predetermined position at the third staging area.

2.   The system of claim 1 wherein said feeder is adapted to return each punched specimen to said first staging area.

3.   The system of claim 2 wherein each biological specimen of said repository is capable of providing a plurality of samples.

4.   The system of claim 2 wherein said robot includes a reader capable of identifying which specimens to retrieve from said repository.

5.   The system of claim 4 wherein said reader is a bar code reader.

6.   The system of claim 1 wherein the biological specimen is blood.

7.   The system of claim 1 wherein the biological specimen is blood serum.

8.   The system of claim 1 wherein the biological specimen is blood plasma.

9.   The system of claim 1 wherein the biological specimen is blood lymphocytes.

1   10.   The system of claim 1 wherein the biological specimen is fixed tissue extracts.

1   11.   The system of claim 1 wherein the biological specimen is unfixed tissue extracts.

1   12.   The system of claim 1 wherein the biological specimen is buccal scrapes.

1   13.   The system of claim 3 wherein the biological specimen is purified DNA.

1   14.   The system of claim 3 wherein the biological specimen is purified RNA.

1   15.   The system of claim 3 wherein the biological specimen is purified protein.

1   16.   A biological retrieval and processing system comprising:
2   a repository of biological specimens;
3   a robot for retrieving predetermined specimens from said repository and delivering said
4   specimens to a first staging area, and for returning said specimens from said first
5   staging area back to the repository;
6   a feeder adapted to select said specimens from said first staging area and selectively remove
7   specific specimens for delivery to a second staging area; and
8   a punch head operatively associated with said feeder adapted to remove a sample from a
9   substrate of each said selectively removed specimens and deliver each said sample
10   to a predetermined position at a third staging area.

1   17.   The system of claim 16 wherein said feeder is adapted to return each punched
2   specimen to said first staging area

1   18.   The system of claim 17 wherein each biological specimen is capable of providing a
2   plurality of samples.

1   19.   A biological retrieval and processing system comprising:
2   a medical database;

3   a repository of biological specimens, each specimen having an identification code and

4   correlated to a medical entry in said medical database;

5   a robot having a reader capable of reading said identification codes and retrieving

6   predetermined specimens from said repository and delivering said retrieved

7   specimens to a first staging area and for returning said specimens back to said

8   repository;

9   a feeder adapted to retrieve said specimens from said first staging area and deliver each

10   retrieved specimen to a second staging area; and

11   a punch head operatively associated with said feeder adapted to remove a sample from a

12   substrate of each retrieved specimen and deliver each said sample to a predetermined

13   position in the third staging area,

14   wherein said feeder returns each punched specimen to said first staging area.

1   20.   A method for collecting selected samples from a repository of biological specimens,

2   comprising the steps of:

3   identifying specimens for retrieval from the repository;

4   retrieving the identified specimens from the repository;

5   delivering the specimens retrieved from the repository to a first staging area;

6   removing selected specimens retrieved from the first staging area and delivering each said

7   selected specimen to a second staging area;

8   removing a biological sample from each specimen at the second staging area; and

9   delivering each biological sample removed from a selected specimen at the second staging

10   area to a third staging area for subsequent processing;

1   21.   The method according to claim 20 wherein the retrieving step is done robotically.

1   22.   The method according to claim 21 wherein the third staging area comprises a

2   multiwell tray providing for the positioning of each sample taken from each specimen into an

3   individual well of said tray and correlatable to the medical records of a particular individual.

1   23.   The method according to claim 20 wherein the method further comprises purifying

2   the individual samples retrieved from each specimen collected in the third staging area for further

3   testing.

1   24.   The method of claim 20 wherein the method also includes identifying which

2   specimens to retrieve from a medical database.

1   25.   The method according to claim 20 wherein the method further comprises purifying

2   and amplifying the individual samples retrieved from each specimen collected in the third strategy

3   area for further testing.

1   26.   An apparatus for preparing biological samples from selected specimens comprising:

2   a feeder assembly adapted to remove a selected substrate from a storage container;

3   a punching assembly having a frame and a movable arm supported by said frame and

4   displaceable in an x, y and z axis relative to said frame;

5   a punch head mounted on the movable arm having

6   a tip adapted to punch a pellet from the substrate,

7   a reservoir adapted to retain the pellet, said reservoir adjacent the tip, and

8   an ejector adapted to eject the pellet from the reservoir; and

9   a position controller adapted to move the arm and position the tip over the substrate in a first

10   position and position the tip over a sample container in a second position,

11   said ejector adapted to eject said pellet in said second position.

1.   27.   The apparatus of claim 26, wherein the punch head also includes a piston having a

2   first end proximate the reservoir and a second end distal the reservoir, and the apparatus further

3   comprises:

4   a rod contacted the second end of the piston; and

5   a solenoid connected to the rod and adapted to actuate the rod.

1   28.   The apparatus of claim 26, wherein the substrate comprises a flexible substrate held

2   in a frame.

1   29.   The apparatus of claim 28, wherein the flexible substrate comprises FTA paper.

1   30.   The apparatus of claim 28, wherein the frame comprises a 35 mm slide frame.

1   31.   The apparatus of claim 26, wherein the storage container comprises a plurality of
2   slots, each adapted to hold a substrate.

1   32.   The apparatus of claim 26 wherein the feeder assembly is further adapted to return
2   the substrate to an original position in the storage container after the substrate is punched.

1   33.   The apparatus of claim 26, wherein the sample container comprises a well in a
2   multiwell tray.

1   34.   The apparatus of claim 26, wherein the substrate comprises indexing indicia, said
2   apparatus further comprising a reader adapted to recognize the indexing indicia.

1   35.   The apparatus of claim 34, wherein the indexing indicia comprises a bar code, and
2   wherein the reader comprises a bar code reader.

1   36.   The apparatus of claim 26 further comprising a slide positioning controller adapted
2   to recognize previously punched areas on the substrate, wherein the position controller is connected
3   to the slide positioning controller and adapted to position the tip over an unpunched area on the
4   substrate in the first position.

1   37.   The apparatus of claim 36, wherein the slide positioning controller includes a
2   microprocessor and an imaging device correlated to said microprocessor.

1   38.   The apparatus of claim 36 further comprising a database comprising data
2   representative of punched or unpunched areas on a substrate corresponding to a particular indexing

3   indicia, wherein the microprocessor is connected to the database and is adapted to position the tip

4   over an unpunched region on the substrate in the first position.

1   39.   An apparatus for preparing biological samples comprising:

2   a punching pad;

3   a feeder for delivery of a specimen, having a substrate, to the punching pad;

4   a punch head having:

5   a tip adapted to punch a pellet from the substrate,

6   a reservoir adapted to retain the pellet, said reservoir adjacent the tip, and

7   means for ejecting the pellet from the reservoir; and

8   a positioning controller to locate the tip over the substrate in a first position and over a

9   sample container in a second position,

10   said ejecting means ejecting said pellet in said second position.

1   40.   The apparatus of claim 39 wherein said feeder is adapted to return the specimen to

2   an original position after the substrate is punched.

1   41.   The apparatus of claim 39, wherein the substrate comprises a flexible substrate held

2   in a frame.

1   42.   The apparatus of claim 39, wherein the specimen comprises indexing indicia, and

2   further comprising means for recognizing said indexing indicia.

1   43.   The apparatus of claim 42 further comprising a second positioning controller for

2   identifying a viable punching area on the substrate.

1   44.   A method for retrieving biological samples for processing comprising the steps of:

2   robotically removing a first DNA specimen from a specimen holder;

3   automatically positioning the first specimen over a punching pad;

4   punching a pellet from the first specimen with the tip of a punch head and retaining the pellet

5   in a reservoir in the punch head;

6   moving the tip over a sample holder; and

7   depositing the tip in the sample holder.

1   45.   The method of claim 44 further comprising:

2   loading the first specimen in the specimen holder after the specimen is punched;

3   removing a second specimen from the specimen holder;

4   positioning the second specimen on the punching pad; and

5   moving the tip over the second specimen.

1   46.   The method of claim 44, wherein the specimen includes a flexible substrate held in

2   a frame.

1   47.   The method of claim 44, wherein the specimen includes indexing indicia.

1   48.   The method of claim 44 wherein after the positioning step, the method also includes:

2   identifying a viable punching area on the specimen by means of a microprocessor; and

3   automatically positioning the tip over the viable punching area.
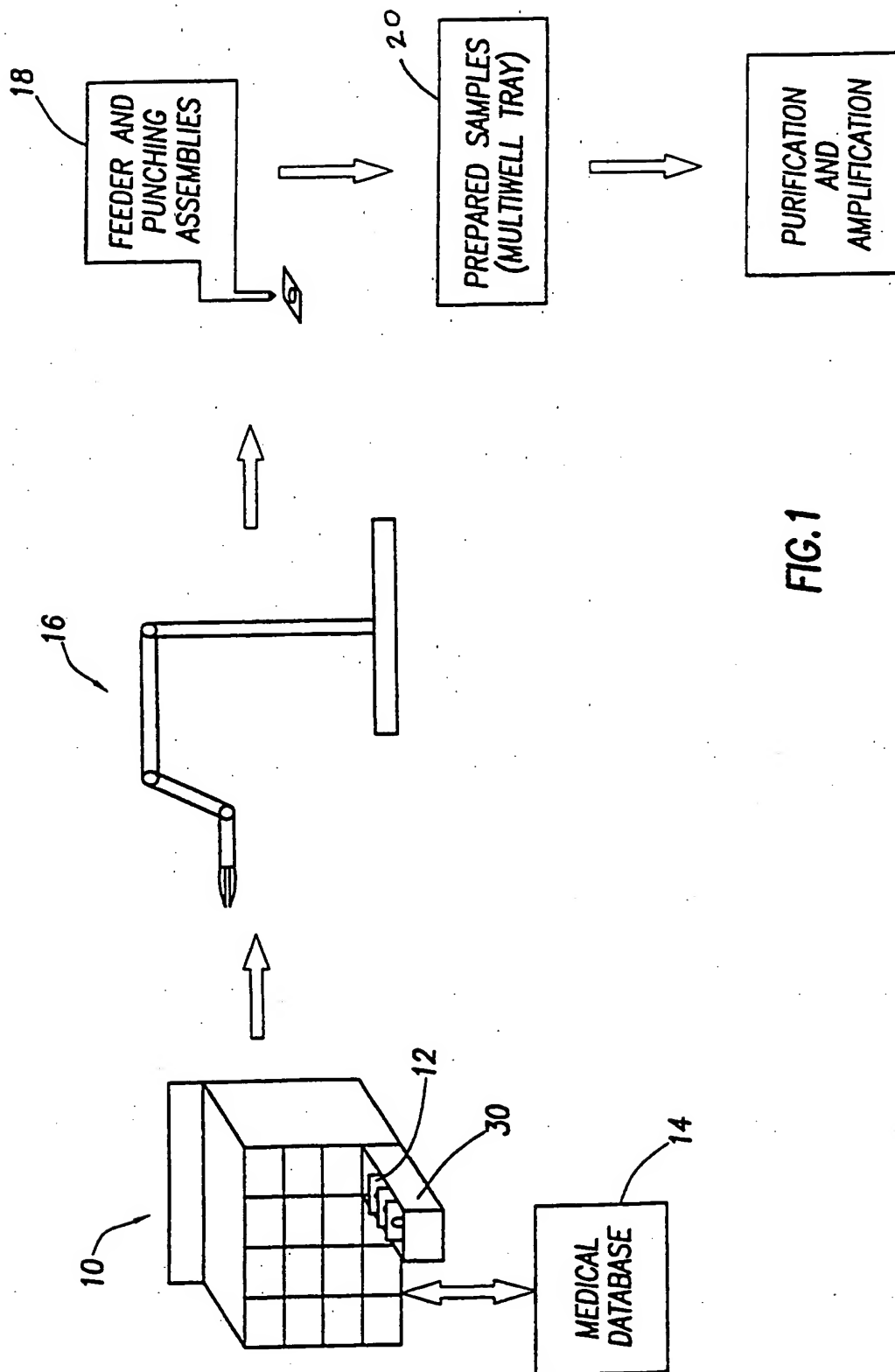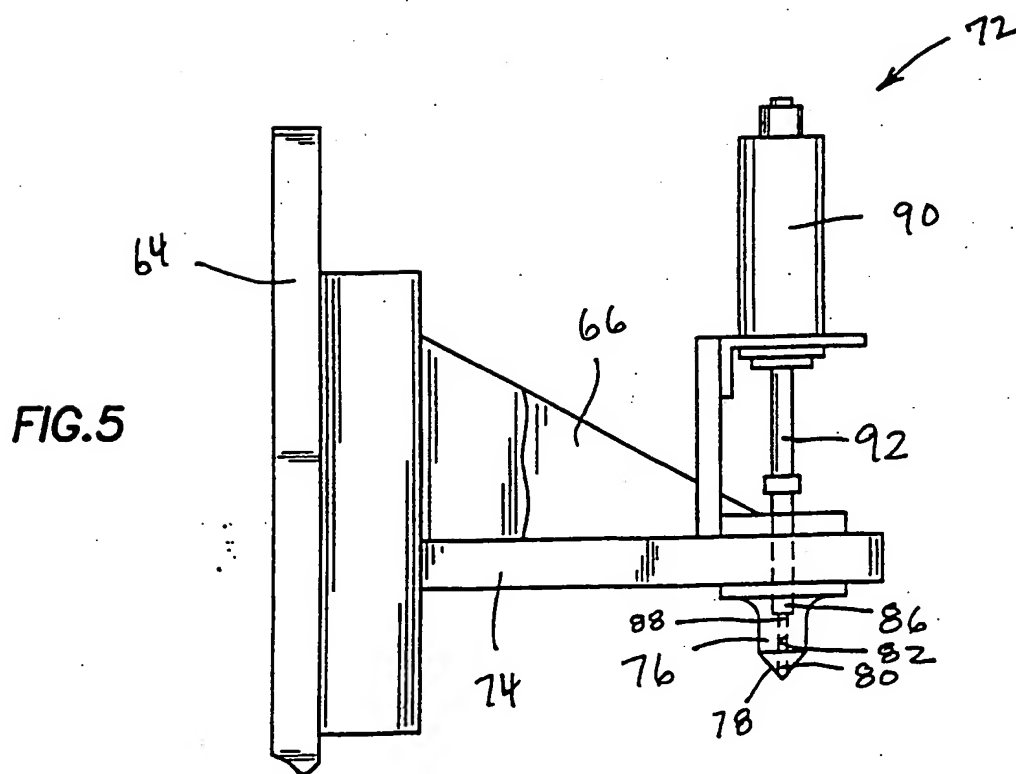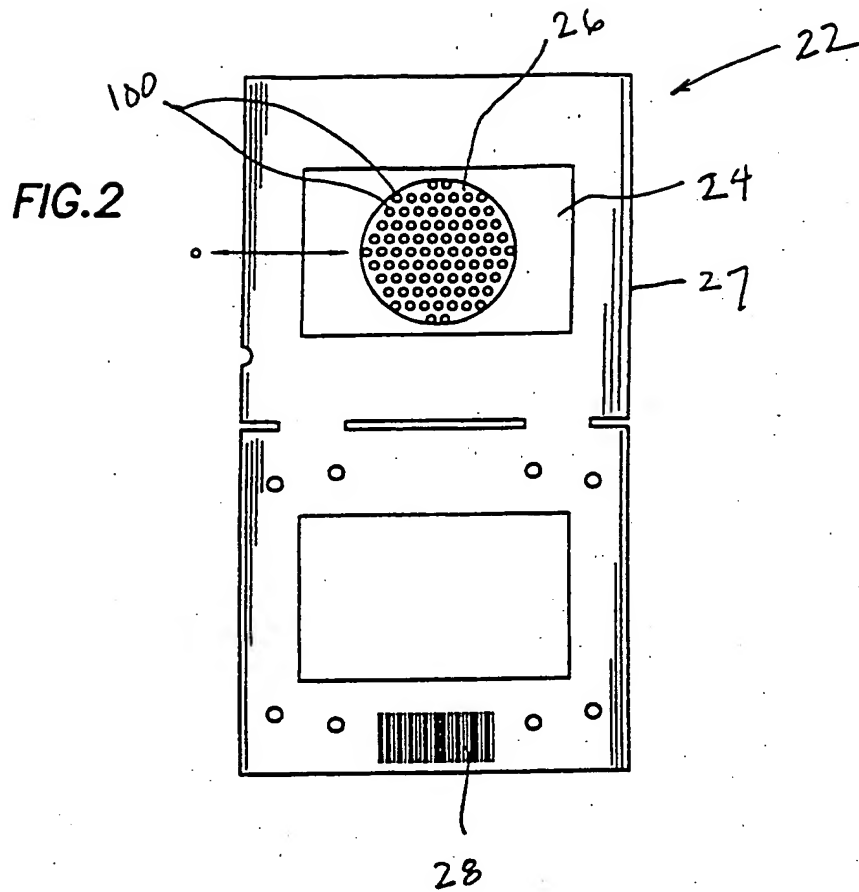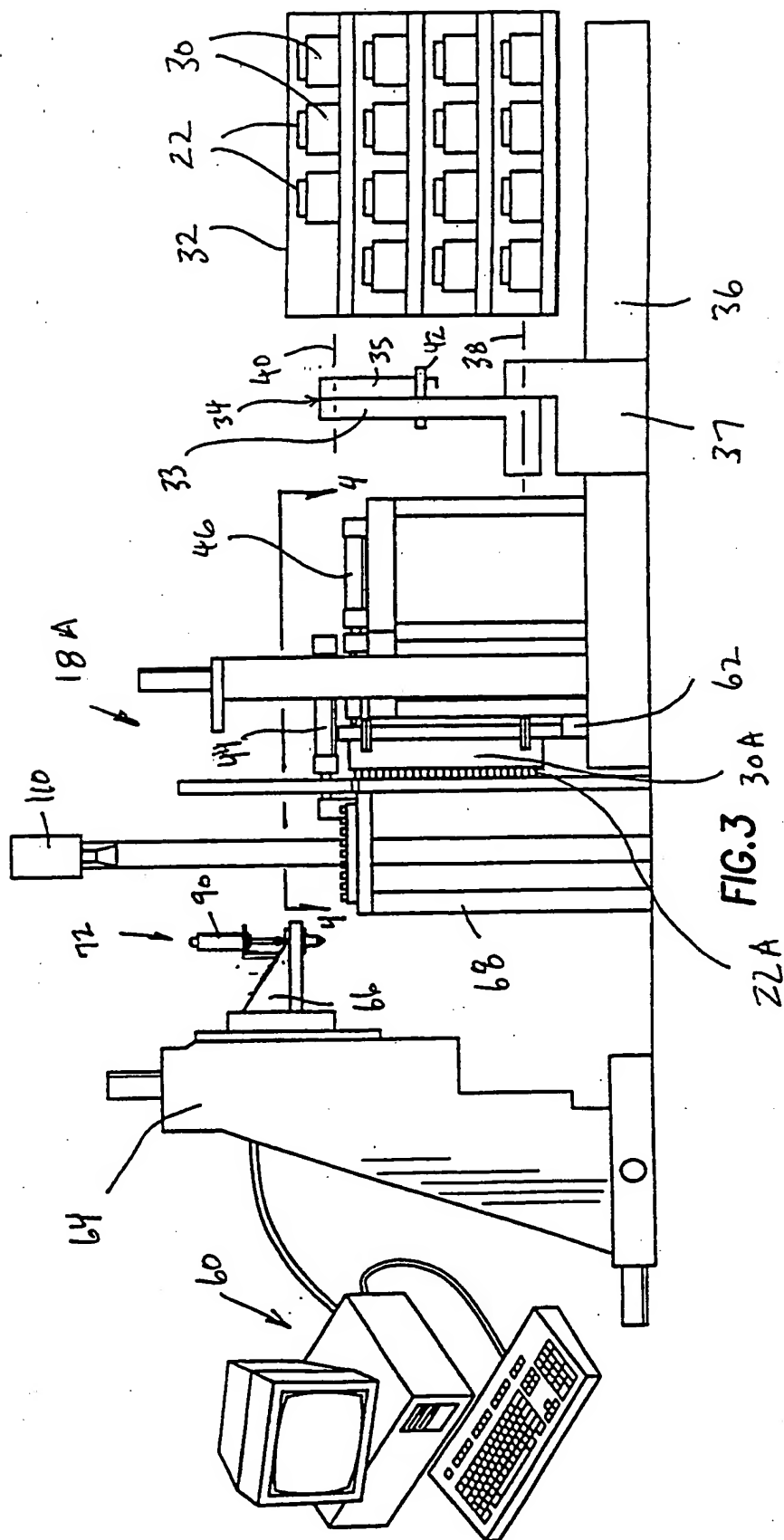
FIG.1

FIG.2

FIG.5

FIG.3

FIG.4

100

90

MICROPROCESSOR ── X,−Y,−Z ──► MOVES ARM 66

MEMORY

FIG.6

150   152

MICROPROCESSOR ──────── READY
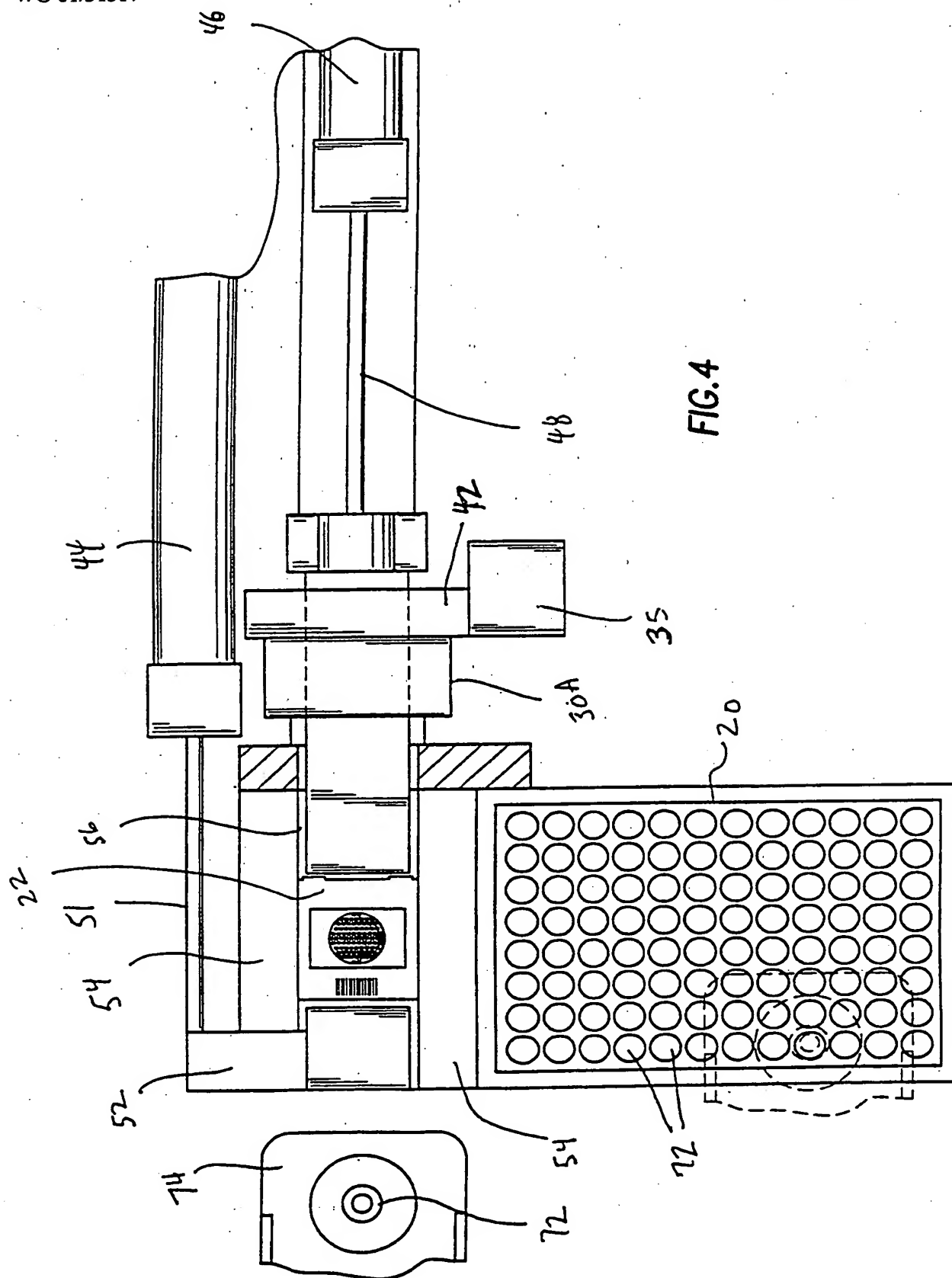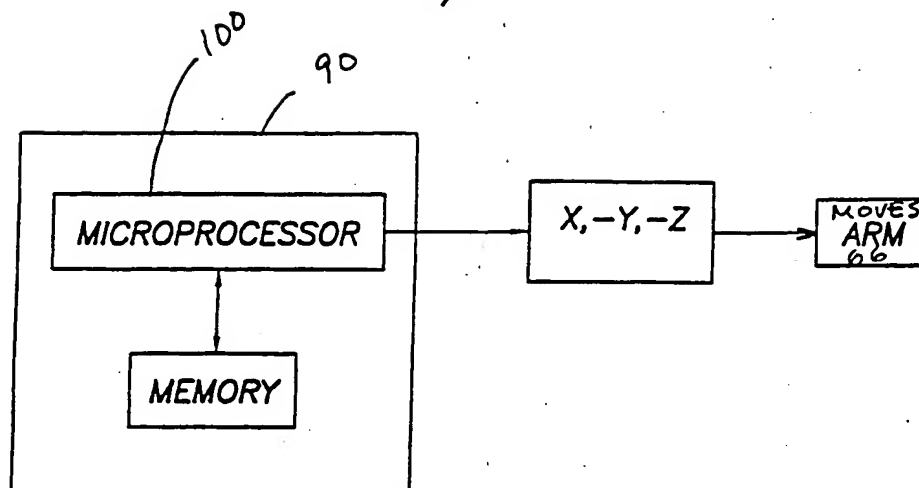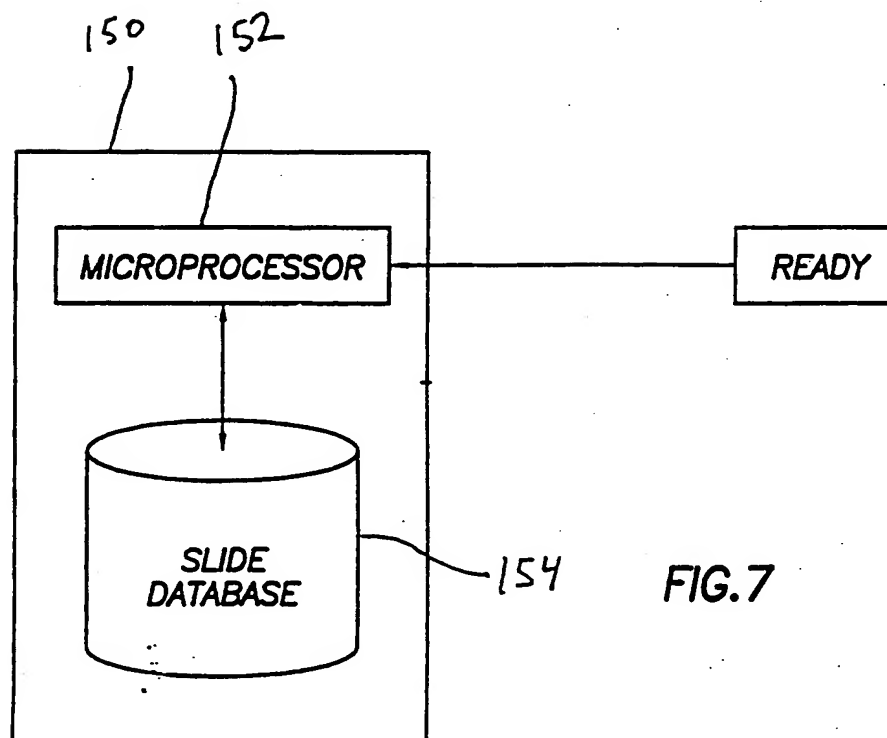
SLIDE DATABASE ── 154   FIG.7

INTERNATIONAL SEARCH REPORT

	International application No.
	PCT/US00/29332

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 21/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/63. 65. 67. 100. 104; 435/ 286.1. 286.2; 436/ 43. 44. 46. 47. 48. 49. 174. 177; 73/864.31. 864.41

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and. where practicable. search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,800,777 A (JEHAN) 01 September 1998, entire document.	1-3,6-9, 16-23, 25, 44-45
X	US 4,684,613 A (BARRERE et al) 04 August 1987, entire document.	26, 39, 44-45
X, P	US 6,103,518 A (LEIGHTON) 15 August 2000, entire document.	26-28, 33, 36, 38-39
Y	US 5,638,170 A (TRINKA et al) 10 June 1997, entire document.	1-48
Y	US 5,460,057 A (OSTRUP et al) 24 October 1995, entire document.	1-48

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 JANUARY 2001	**23 FEB 2001**
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington. D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PATRICIA K. BEX Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998) *

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/63, 65, 67, 100, 104; 435/ 286.1, 286.2; 436/ 43, 44, 46, 47, 48, 49, 174, 177; 73/864.31, 864.41